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The influence of blood meals on accumulation of arachidonic acid by adult stable flies

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We investigated the influence of blood meals on accumulation of the polyunsaturated fatty acid (PUFA), 20:4n-6, by adult stable flies, *Stomoxys calcitrans* (L.). The fatty acid compositions of phospholipids (PLs) and triacylglycerols (TGs) prepared from adult male and female stable flies that had been maintained either with or without blood meals were determined, and incorporation of exogenous radioactive fatty acids into tissue lipids of adults was monitored. The major PL components were 16:0, 16:1, 18:1 and 18:2n-6. Two C_{20} PUFAs, 20:4n-6 and 20:5n-3 were associated with PLs in the 1–5% range. The major TG components were 14:0 and 16:1. Although absence of the normal adult blood meals influenced the overall fatty acid profiles of male and female stable flies, proportions of C_{20} PUFAs in PLs of blood-fed flies were not substantially higher than proportions of C_{20} PUFAs in their sucrose-fed counterparts. The fatty acid incorporation studies indicate that the ability to incorporate exogenous fatty acids is decreased in older adult stable flies. We conclude that the larval stages of stable flies are more important for accumulation of C_{20} PUFAs than the adult stages.

Key words: Stable flies; *Stomoxys calcitrans*; Blood meals; Phospholipids; Fatty acids; Arachidonic acid; Eicosanoids.

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Introduction

Eicosanoid is a general term for all oxygenated metabolites of arachidonic and certain other C_{20} polyunsaturated fatty acids (PUFAs). The structures of C_{20} PUFAs, eicosanoids, and their biosynthetic pathways, are illustrated in several reviews (Stanley-Samuelson 1987, 1991, 1993, 1994). Although these compounds are well appreciated in terms of their clinical significance in human and veterinary medicine, there is increasing recognition of the physiological and ecological significance of prostaglandins (PGs) and related eicosanoids in insects and other invertebrates. PGs release egg-laying behavior in some, but certainly not all, insect species (Stanley-Samuelson and Loher, 1986). PGs and other eicosanoids mediate cellular immune responses to bacterial infections in insects, and possibly other invertebrates (Stanley-Samuelson *et al.*, 1991). PGs are also elements in the regulation of basal fluid secretion rates in Malpighian tubules of mosquitoes (Petzel and Stanley-Samuelson, 1992). For a single example, the behavior of penetrating mammalian skin by

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cercarial larvae of the schistosome *Schistosoma mansoni* is mediated by eicosanoids (Salafsky and Fusco, 1987). We suggest that the significance of these compounds in the biology of invertebrates will gain increasing recognition.

The biosynthesis of eicosanoids depends upon availability to eicosanoid biosynthetic enzymes of any of three eicosanoid precursor PUFAs, 20:3n-6, 20:4n-6 and 20:5n-3. On the contemporary models, these compounds are associated with cellular phospholipids (PLs), from whence they are hydrolyzed for eicosanoid biosynthesis by action of phospholipase A_2 (Stanley-Samuelson, 1994). These fatty acids may be biosynthesized by elongation/desaturation of dietary C_{18} PUFAs (Stanley-Samuelson *et al.*, 1988) or they may be acquired from the diet. In general, hematophagous arthropods may accumulate C_{20} PUFAs from their blood meals, which are rich in these components. To gain more information on the dietary sources of C_{20} PUFAs in hematophagous insects, we considered the accumulation of 20:4n-6 from blood meals in adult stable flies, *Stomoxys calcitrans* (L.). Here we report that proportions of 20:4n-6 in blood-fed and sugar-fed stable flies were similar, from which we suggest that stable flies accumulate C_{20} PUFAs primarily during larval stages rather than from the blood meals taken in adulthood.

Materials and Methods

Insects

Stable flies, *S. calcitrans*, are maintained in routine culture in the Midwest Livestock Insects Research Laboratory (USDA/ARS, Lincoln, NE, U.S.A). Adults are kept in screened cages (about 46 cm^3, approximately 2000 flies/cage) in a colony room maintained at about 25°C and 50% RH. The adults are fed once daily from 7×30 cm cotton pads saturated with citrated bovine blood. The flies mate randomly, and egg collection begins on day 5. The oligidic larval media is prepared by completely mixing four ingredients (2 l wheat bran, 2 l wood chips (small animal bedding, purchased from Hill Hatchery, Lincoln, NE, U.S.A.), 236 ml fish meal (Service Supply Feed Co., Lincoln, NE, U.S.A.) and 1.6 l water) in a large pan. One milliliter of eggs, about 10,000 eggs/ml, are placed in the containers of larval media. Pupae are harvested from the larval media about 10–14 days later, and transferred to cages for adult emergence and maintenance. Some adults were not provided with blood meals. Pupae were transferred to adult emergence cages, and the adults were provided with water (in a cup filled with styrofoam beads and water) and dry sucrose.

At selected ages, the adults were used in experiments on the day they were taken from the colony room. To assess the possibility that the blood in the alimentary canal of adult flies influenced the fatty acid profiles of blood-fed flies, some of the blood-fed flies were anesthetized by chilling, and the alimentary canals were removed. These are called "gut-free" flies in this report.

Lipid extraction and analysis

We followed the routine protocols described elsewhere (Stanley-Samuelson *et al.*, 1990; Ogg *et al.*, 1991). Total lipid extracts were prepared by homogenizing groups of 5 adult male or female stable flies in 3 ml of chloroform:methanol (2:1, v/v). Autoxidation of PUFAs was minimized by adding 50 μl of butylated hydroxytoluene (2%, w/v in chloroform) to the extraction system.

For analysis of phospholipid (PL) and triacylglycerol (TG) fatty acids, total lipid extracts were dried under a stream of N_2, then applied to thin-layer chromatography (TLC) plates (silica gel G, 20×20 cm, 0.25 mm thick; Sigma Chemical Co., St Louis, MO, U.S.A.). The plates were developed in petroleum ether:diethyl ether:acetic acid (80:20:1, v/v). Bands that corresponded in R_f to PL and TG standards were scraped into reaction tubes, and the fatty acids in each fraction were transmethylated to fatty acid methyl esters (FAMEs) by refluxing the samples in acidified methanol for 90 min (Stanley-Samuelson and Dadd, 1983).

Gas chromatography and gas chromatography–mass spectrometry

The FAMEs were extracted three times from the reaction mixtures with petroleum ether, concentrated, and then analyzed as described by Howard and Stanley-Samuelson (1990). FAMEs were chromatographed isothermally at 190°C on a Hewlett-Packard HP-5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with a SP-2330 capillary column (0.25 mm × 30 m, 0.2 μm film thickness, Supelco, Bellefonta, PA, U.S.A.), a flame-ionization detector, and a HP-3396A recording integrator. Injections were made in split mode (45:1) and separations were carried out with He_2 carrier gas at 0.6 ml/min. FAMEs were tentatively identified by comparisons of retention times with authentic standards from Sigma.

The tentative identifications were confirmed by gas chromatography–mass spectrometry. Analyses were conducted on a HP 5790 gas chromatograph equipped with a 0.25 mm by

30 m Supelcowax 10 capillary column (Supelco). The gas chromatograph was interfaced with a HP 5970 electron impact mass selective detector operated at 70 eV. Chromatographic conditions included a 45-s splitless injection, a 2-min hold period and 1°C/min temperature program from 170 to 200°C. Ultrapure He_2 was the carrier gas at 1 ml/min. Retention times and total ion mass spectra of FAMEs were compared with authentic standards from Sigma.

Incorporation of exogenous radioactive fatty acids into stable fly lipids

Two radioactive fatty acids, arachidonic acid ([5,6,8,9,11,12,14,15-^{3}H]-20:4n-6, specific activity 240 Ci/mmol) and oleic acid ([9,10-^{3}H]18:1n-9, specific activity 10 Ci/mmol), were purchased from New England Nuclear (Cambridge, MA, U.S.A.). Each fatty acid was appropriately diluted, then dissolved to 0.2 μCi/μl in Tween-80 (0.5 mg/ml). Incubations were started by injecting 1.0 μl of a fatty acid into the thorax of each adult stable fly (age 1 or 5 days, as indicated in Results). Injections were performed under a dissecting microscope, using a Hamilton 701 syringe equipped with a 22 gauge needle. The flies were anesthetized by chilling on ice, then mounted upon an arena made of modelling clay. The flies were mounted ventrum down, such that the dorsal aspect of the thorax was exposed. The needle was inserted into the anterior portion of the prothorax, which was exposed by tilting the head forward. After 2-hr incubations at room temperature, the flies were processed for total lipid extraction, as described above.

The total lipid extracts were resolved into PLs, TGs diacylglycerols (DGs) and free fatty acids (FFA) using the TLC system described above. The radioactivity in each fraction was determined by scanning the plates with a BioScan 200 Imaging Scanner and by scraping the bands that corresponded to PL and TG into liquid scintillation vials. Five milliliters of scintillation cocktail (Ecolite (+), ICN, Costa Mesa, CA, U.S.A.) were added to each vial, and the radioactivity in each fraction was estimated by liquid scintillation counting on a LKB-Wallac 1209 Rack beta A liquid scintillation counter (Helsinki, Finland) at 50% counting efficiency for tritium.

Results

Fatty acid compositions

Fatty acid compositions of PLs prepared from 2-, 6- and 10-day-old adult stable flies that were maintained on blood, on sugar, and from 6-day-old blood-fed, gut-free stable flies are presented in Table 1 for males and Table 2 for females. The major components were 16:0, 16:1, 18:1n-9, and 18:2n-6. Two C_{20} PUFAs, 20:4n-6 and 20:5n-3 were present in the 1–5% range. The overall patterns fit into the general profiles of dipteran PL fatty acid compositions (Fast, 1970; Stanley-Samuelson *et al.*, 1988),

Table 1. Fatty acid composition, as proportions of total fatty acids, in phospholipids prepared from total extracts of adult male stable flies at indicated ages

Fatty acids	2-day		6-day		10-day		Gut-removed insects
	Blood	Sugar	Blood	Sugar	Blood	Sugar	
13:0	0.1	0.26 (0.2)	1.2 (0.18)	0.53 (0.59)	0.19 (0.1)	1.35 (0.35)	1.63 (0.84)
14:0	0.11 (0.06)	0.82 (0.1)	4.51 (1.2)	1.2 (0.26)	1.28 (0.4)	3.5 (1.4)	2.3 (1.4)
15:0	0.11 (0.01)	0.3 (0.02)	0.12	0.37 (0.04)	0.71 (0.3)	2.9 (0.5)	1.28 (0.7)
16:0	14.05 (0.76)	15.03 (0.61)	13.89 (0.4)	14.03 (0.7)	12.45 (0.7)	18.3 (2.7)	15.56 (1.6)
16:1	30.76 (2.4)	15.33 (0.57)	33.82 (4.6)	25.3 (2.6)	37.63 (2.6)	21.83 (2.1)	26.13 (3.8)
17:0	0.7 (0.13)			0.1 (0.0)	0.19	3.2 (0.71)	
18:0	2.15 (0.21)	2.77 (0.12)	1.81 (0.53)	3.4 (0.26)	1.59 (0.2)	4.0 (1.3)	2.49 (0.8)
18:1n-9	25.11 (0.04)	24.1 (0.61)	20.94 (1.7)	24.5 (0.2)	24.17 (0.9)	19.4 (4.2)	24.92 (0.56)
18:2n-6	13.93 (1.3)	25.63 (0.38)	11.26 (0.5)	20.13 (0.6)	11.93 (0.6)	9.6 (2.8)	12.64 (0.69)
18:3n-6	1.51 (0.2)	0.66 (0.05)	2.21 (1.4)	0.48 (0.01)	0.45 (0.2)		2.55 (1.2)
18:3n-3		2.63 (0.15)	1.42 (0.6)	2.0 (0.1)	1.91 (1.3)	3.1 (0.62)	
18:4n-3							
19:0							
20:0	0.7 (0.03)		1.13 (0.2)		0.69 (0.3)		
20:1							
20:3n-6							
20:4n-6	1.19 (0.06)	0.65 (0.04)	1.25 (0.3)	0.52 (0.02)	2.26 (0.4)		0.73 (0.12)
20:5n-3	5.6 (0.21)	8.5 (0.52)	3.65 (0.6)	6.13 (0.25)	4.11 (0.2)	4.8 (0.46)	5.98 (0.8)
21:0							
22:0							
22:1							
22:2n-6							
24:1							

Values are means of three to four separate analyses (SD).

Table 2. Fatty acid composition, as proportions of total fatty acids, in phospholipids prepared from total extracts of adult female stable flies at indicated ages

Fatty acids	2-day		6-day		10-day		Gut-removed insects
	Blood	Sugar	Blood	Sugar	Blood	Sugar	
13:0	0.14 (0.11)	0.05 (0.03)	0.93 (0.57)	0.68 (0.31)	0.83 (0.6)		
14:0	0.9 (0.11)	0.83 (0.03)	6.17 (2.3)	0.63 (0.31)	3.32 (2.4)	0.8 (0.15)	1.36 (0.91)
15:0	0.25 (0.06)	0.34 (0.06)		0.31 (0.0)	1.53 (0.9)	0.27 (0.03)	0.76 (0.53)
16:0	15.69 (0.47)	16.83 (0.12)	17.47 (6.9)	13.73 (0.32)	11.75 (0.3)	12.2 (0.61)	15.3 (0.57)
16:1	21.96 (1.7)	16.4 (1.2)	31.03 (6.5)	22.77 (1.8)	40.17 (1.3)	32.7 (2.8)	28.91 (3.6)
17:0	0.72 (0.44)		0.42		0.09	0.46 (0.0)	
18:0	2.2 (0.02)	2.33 (0.06)	2.0 (0.5)	3.27 (0.12)	1.33 (0.3)	5.33 (0.15)	1.87 (0.11)
18:1n-9	26.59 (0.5)	24.3 (0.36)	21.48 (1.6)	26.1 (0.36)	24.35 (1.4)	28.57 (0.12)	25.09 (1.3)
18:2n-6	16.95 (0.5)	23.97 (0.93)	10.16 (0.3)	20.53 (1.0)	9.55 (0.7)	11.8 (0.72)	12.7 (0.28)
18:3n-6	1.52 (0.04)	0.67 (0.06)	0.84 (0.5)	0.5 (0.05)	0.47 (0.2)	0.8 (0.0)	1.74 (1.0)
18:3n-3		2.47 (0.21)	1.83 (0.1)	2.03 (0.12)	1.34 (0.7)	1.3 (0.1)	
18:4n-3							
19:0							
20:0	0.82 (0.09)		0.92 (0.3)		0.69 (0.1)		
20:1							
20:3n-6							
20:4n-6	0.83 (0.1)	0.63 (0.04)	1.33 (0.2)	0.59 (0.03)	1.71 (0.3)		0.68 (0.25)
20:5n-3	7.51 (0.14)	7.53 (0.12)	2.99 (0.2)	6.9 (0.36)	2.21 (0.9)	7.03 (0.4)	6.7 (0.65)
21:0							
22:0							
22:1							
22:2n-6							
24:1							

Values are means of three to four separate analyses (SD).

with high proportions of 16:1 and relatively low proportions (<30%) of 18:2n-6. The fatty acid profiles of 6- to 7-day-old blood-fed, gut-free stable flies were fairly similar to the profiles of their intact counterparts, indicating that the fatty acids that are present in substantial amount in the mammalian blood in the guts of intact animals do not change the outcome of the PL fatty acid analyses.

Slightly different fatty acid profiles were associated with the age of the adult stable flies, their sex and the absence of their normal blood meals (Tables 1 and 2). For males, the proportions of 16:1 associated with PLs increased from 31% at day 2 to 38% at day 10. The change was more pronounced for females, in which PL 16:1 increased from 22% at day 2 to 40% at day 10. These changes in 16:1 are reflected in smaller changes in proportions of other PL fatty acids. The absence of blood meals also influenced the fatty acid profiles. Compared to their blood-fed counterparts, sucrose-fed males and females were lower in PL 16:1 and higher in PL 18:2n-6.

Fatty acid compositions of TGs prepared from 2-, 6- and 10-day-old adult stable flies that were maintained on blood and on sugar, and from 6-day-old blood-fed, gut-free stable flies are presented for males and females in Tables 3 and 4, respectively. The TG patterns differed from most insects, with high proportions of 14:0. Characteristically high proportions of 16:1 were also present in TGs. As is usual for insects, only very low proportions of PUFAs were associated with TGs. The fatty acid profiles were fairly similar for males and females, particularly at days 6 and 10. The profiles changed considerably as the flies aged. TGs from day 2 flies had very high proportions (>50%) of 14:0, which decreased to about 18% by day 6, then increased to 24% by day 10. These changes were reflected in similar changes in proportions of TG 16:1, which approximately doubled from day 2 to day 6, and slightly decreased by day 10. In contrast to the PLs, the fatty acid profiles of the TGs were influenced by the presence of blood in the guts of male and female flies. The TGs from 6- to 7-day-old gut-free stable flies more closely matched the TG profiles of day 2 intact flies, with 40–50% 14:0. The absence of the usual blood meals also influenced the TG fatty acid profiles. Compared to their blood-fed counterparts at all ages, sucrose-feeding resulted in much higher proportions of 14:0, and considerably reduced proportions of 16:1.

Incorporation of radioactive fatty acids into tissue lipids

Stable flies are able to incorporate radioactive fatty acids into tissue lipids; however, patterns of incorporation were influenced by sex, age and fatty acid structure (Fig. 1). About 90% of the radioactivity from 20:4n-6 recovered from day 1 adults was incorporated into lipids, the remainder was recovered as unesterified fatty

Table 3. Fatty acid composition, as proportions of total fatty acids, in triacylglycerols prepared from total extracts of adult male stable flies at indicated ages

Fatty acids	2-day		6-day		10-day		Gut-removed insects
	Blood	Sugar	Blood	Sugar	Blood	Sugar	
13:0	0.51 (0.22)	0.08 (0.3)	0.26	0.6 (0.0)	0.29 (0.11)	0.69 (0.4)	1.27 (0.62)
14:0	58.6 (10.1)	89.2 (0.26)	17.94 (1.9)	42.8 (5.4)	23.68 (1.0)	68.6 (3.6)	41.68 (6.6)
15:0	1.8 (1.4)	0.03 (0.0)	4.33 (0.04)	1.17 (0.35)	2.83 (0.4)	0.5 (0.0)	1.01 (1.2)
16:0	15.68 (2.9)	3.17 (0.06)	28.69 (1.6)	20.8 (2.1)	30.42 (0.3)	11.1 (1.9)	19.43 (1.2)
16:1	15.78 (4.9)	1.87 (0.21)	34.01 (1.5)	15.9 (1.6)	29.82 (0.9)	5.8 (0.5)	18.94 (2.1)
17:0	0.47 (0.1)	0.09 (0.0)			0.06		
18:0	0.61 (0.1)	0.2 (0.0)	1.46 (0.3)	3.77 (0.25)	1.49 (0.3)	2.6 (0.6)	0.99 (0.01)
18:1n-9	5.43 (1.0)	1.83 (0.31)	11.36 (2.3)	8.7 (0.7)	10.04 (0.6)	6.9 (1.4)	10.98 (0.84)
18:2n-6	0.76 (0.2)	1.37 (0.15)	1.97 (1.4)	1.33 (0.25)	0.8 (0.05)	0.72 (0.4)	2.18 (0.81)
18:3n-6							1.59 (0.98)
18:3n-3			0.39	0.3 (0.2)		2.3 (0.57)	
18:4n-3							
19:0							
20:0							
20:1							
20:3n-6							
20:4n-6							
20:5n-3	0.23	0.12 (0.0)					
21:0							
22:0							
22:1							
22:2n-6							
24:1							

Values are means of three to four separate analyses (SD).

acids. Most of the recovered radioactivity (54%) was associated with TGs in females, and with DGs (46%) in males. Lower proportions of the recovered radioactivity was associated with PLs, about 22% for females and 13% for males. In contrast to day 1 adults, about 80% of the radioactivity associated with 20:4n-6 was recovered from day 5 adults as free fatty acid. Moreover, the incorporation patterns were similar for males and females, with about 12% recovered from PLs, and very little from neutral lipids.

To consider the effects of fatty acid structure

Table 4. Fatty acid composition, as proportions of total fatty acids, in triacylglycerols prepared from total extracts of adult female stable flies at indicated ages

Fatty acids	2-day		6-day		10-day		Gut-removed insects
	Blood	Sugar	Blood	Sugar	Blood	Sugar	
13:0	0.13 (0.06)	0.3 (0.2)	0.03 (0.02)	0.5 (0.12)	1.34 (0.78)		0.13 (0.03)
14:0	74.27 (7.07)	68.6 (1.7)	18.69 (0.36)	37.8 (13.8)	23.79 (0.5)	83.9 (16.9)	50.47 (3.2)
15:0	0.54 (0.1)	0.24 (0.23)	4.72 (0.3)	1.03 (0.4)	4.28 (0.95)		1.05 (1.4)
16:0	9.02 (3.0)	8.9 (0.1)	27.67 (0.9)	25.4 (6.9)	26.63 (0.9)	5.6 (6.8)	17.4 (0.56)
16:1	6.53 (2.6)	5.5 (0.1)	36.9 (0.9)	16.4 (2.0)	31.72 (1.7)	4.8 (6.1)	15.56 (2.8)
17:0	0.91 (0.09)	1.4 (0.69)	0		0		
18:0	0.53 (0.2)	0.63 (0.21)	1.32 (0.3)	5.03 (0.9)	0.96 (0.06)	1.47 (1.5)	1.01 (0.06)
18:1n-9	5.01 (0.9)	6.17 (1.27)	9.52 (0.5)	10.7 (2.8)	8.14 (0.2)	3.22 (3.2)	10.78 (2.7)
18:2n-6	1.42 (0.23)	3.5 (0.26)	0.94 (0.1)	1.7 (0.69)	1.0 (0.06)	0.24 (0.05)	1.56 (0.23)
18:3n-6							
18:3n-3		0.5 (0.57)		0.39 (0.36)			
18:4n-3							
19:0							
20:0							
20:1							
20:3n-6							
20:4n-6					0.11 (0.01)		
20:5n-3	0.23 (0.05)	0.4 (0.05)	0.07		0.08		0.39
21:0							
22:0							
22:1							
22:2n-6							
24:1							

Values are means of three to four separate analyses (SD).

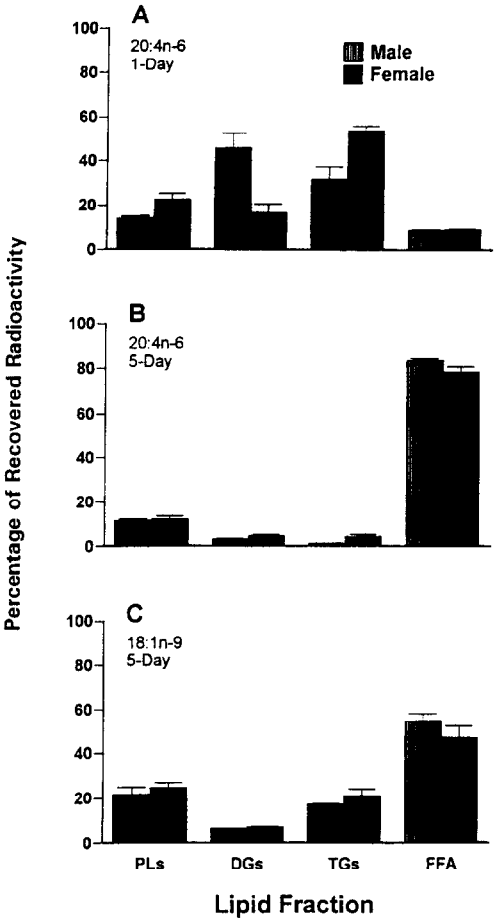

Fig. 1. *In vivo* incorporation of radioactive fatty acids into tissue lipids of adult stable flies, *S. calcitrans*. Radioactive fatty acids were injected into adult flies, and after 2 hr incubations, total lipids were extracted. Lipid fractions were resolved on TLC, and radioactivity was assessed in each fraction by liquid scintillation counting. Each column represents the mean of three experiments, and the vertical error bars indicate 1 SD. Panels A and B represent data for incorporation of arachidonic acid by 1- and 5-day-old adults, and panel C represents data for incorporation of oleic acid by 5-day-old adults.

on incorporation patterns, radioactive 18:1n-9 was injected into day 5 males and females. Again, results from males and females were similar. About half of the radioactivity was recovered as free fatty acid. The incorporated radioactivity was approximately equally distributed in PLs and TGs (*ca.* 20% in each fraction), and 6–7% was recovered from DGs.

Discussion

This report documents the fatty acid compositions of PLs and TGs from adult stable flies, *S. calcitrans*. The major PL components were 16:0, 16:1, 18:1 and 18:2n-6, and the major TG components were 14:0, 16:0, 16:1 and 18:1. Given the exception of 14:0, these profiles fit into the general background of dipteran fatty acid compositions (Fast, 1970). Most of the dipterans that have been analyzed include 14:0 in their tissue lipids, however, it is usually a relatively minor component. In this regard, the stable fly TGs appear more similar to the fatty acid patterns of some Hemiptera, in which 14:0 is commonly a major component of tissue lipids.

As a group, the Diptera stand out from the general background of insect PL and fatty acid compositions (Fast, 1970; Stanley-Samuelson *et al.*, 1988). Unlike most animals, in which choline-containing PLs predominate over ethanolamine-containing PLs, the Diptera often feature very high proportions of ethanolamine-containing PLs. The Diptera also differ in fatty acid compositions, particularly in the characteristically high proportions of 16:1. Relatively low proportions of 16:1 in PL of the syrphid fly *Microdon albicomatus* marks an exception, which appears to be associated with life history. This species is an inquiline predator found in ant colonies, upon which it feeds exclusively (Stanley-Samuelson *et al.*, 1990). So far, the only insect species outside of the Diptera known to feature high proportions of 16:1 are chinch bugs, *Blissus* spp. (Lygaeidae; Hemiptera; Spike *et al.*, 1991).

We also document the presence of C_{20} PUFAs in the stable fly lipids. The biological significance of these components is linked to their roles in cellular membrane structure, and also to serve as substrate for biosynthesis of eicosanoids through oxygenation pathways. On the general background, C_{20} PUFAs are not usually recorded in analyses of insect, and especially dipteran, fatty acids (Stanley-Samuelson and Dadd, 1983). These components were not included in reports on the olive fruit fly, *Dacus oleae* (Madariaga *et al.*, 1974), on the Mediterranean fruit fly, *Ceratitis capitata* (Madariaga *et al.*, 1972), in several studies of the house fly, *Musca domestica* (Fast, 1970), nor in *Drosophila melanogaster* (Rapport *et al.*, 1984; Larsen-Rapport, 1986). In comparison to these species, it might be thought, on the knowledge that mammalian blood is rich in 20:4n-6, that hematophagous flies commonly feature C_{20} PUFAs in their lipids. However, several reports of adult mosquito fatty acids did not record these components (Fast and Brown, 1962; Takata and Harwood, 1964; Van Handel, 1966; Buffington and Zar, 1968; Miller and Novak, 1985). Dadd's discovery that mosquitoes require dietary 20:4n-6, or certain structurally related PUFAs during the larval stages (Dadd and Kleinjan, 1979), pressed the need for more detailed studies of mosquito, and other insect, fatty acid compositions. Stanley-Samuelson and Dadd (1981) detected 20:4n-6 in adult mosquitoes, *Culex*

pipiens, and demonstrated that its occurrence in adult mosquitoes quantitatively depended upon dietary supplies during the larval stages.

Based on comprehensive surveys of literature and detailed chemical analyses, Stanley-Samuelson and Dadd (1983) put forth the idea that C_{20} PUFAs were universally present in insect tissue lipids. Upon finding prostaglandins in the reproductive and other tissues of the house fly, Wakayama *et al.* (1985) re-examined house fly lipids. They confirmed the presence, in very low proportions (0.04%), of 20:4n-6 in PLs, but not neutral lipids. In a similar vein, Pages *et al.* (1986) described eicosanoid biosynthesis in *D. melanogaster*, from which we suggest that traces of 20:4n-6 are probably present in this species, as well. In recent years, we have shown that C_{20} PUFAs are present in very low proportions in whole animal and in specific tissues of several terrestrial insect species, including the mealworm beetle, *Tenebrio molitor*, the desert cicada, *Tibicen dealbatus*, and the tobacco hornworm, *Manduca sexta* (Howard and Stanley-Samuelson, 1990; Stanley-Samuelson *et al.*, 1990; Ogg and Stanley-Samuelson, 1992). These findings, together with recent advances in our understanding of the biological significance of eicosanoids in insects and other invertebrates (Stanley-Samuelson, 1993, 1994), bolster the idea that C_{20} PUFAs are, indeed, universally present in insects. However, the point may be controverted because Dillwith *et al.* (1993), using very deliberate methodologies, were unable to detect even traces of these components in the tissue lipids of aphids. We do not know if aphids naturally biosynthesize prostaglandins. If they do, it would be supposed that at least catalytic levels of C_{20} PUFAs are present in aphid tissues.

Let us turn, now, to possible sources of C_{20} PUFAs in insects. These fatty acids would not occur in the diets of strictly phytophagous species. However, like vertebrates, most insects are probably able to synthesize C_{20} PUFAs from the C_{18} counterparts that occur in nearly all insect foods by elongation/desaturation reactions. These pathways have been described for some insect species, currently thought to represent the class (Stanley-Samuelson *et al.*, 1988). Beyond this, a number of insects, mainly hemimetabolous species, are able to synthesize members of the n-6 family of PUFAs *de novo* (Stanley-Samuelson *et al.*, 1988; Blomquist *et al.*, 1991). This represents a radical departure from the vertebrate background because it is believed that no vertebrate animal is capable of inserting a double bond in the Δ12 position of 18:1n-9. Finally, aquatic insects, as well as species that eat vertebrate animal products will obtain C_{20} PUFAs in their diets.

Blood-feeding arthropods can accumulate substantial proportions of 20:4n-6 from blood meals. Shipley *et al.* (1993) reported that in salivary glands of female ticks *Amblyomma americanum* 20:4n-6 increased from 2% to >8% of the PL fatty acids during the adult feeding phase. On the other hand, the proportions of C_{20} PUFAs in adult mosquitoes appear to be determined on the basis of larval, rather than adult, diets (Stanley-Samuelson and Dadd, 1981, 1983; Dadd *et al.*, 1987, 1988). The fatty acid components of larval diets can also influence the proportions of C_{20} PUFAs in adults by providing substrate for biosynthesis via elongation/desaturation pathways. This was reported for the waxmoth, *Galleria mellonella* (Stanley-Samuelson and Dadd, 1984). Waxmoth larvae were reared on artificial media that provided varying levels of 18:3n-3. The adults of these larvae had proportions of PL 20:5n-3 that were quantitatively determined by the amounts of 18:3n-3 in the culture media. Adult stable flies also maintain C_{20} PUFAs that were accumulated during their larval stages. Fish meal is a major source of dietary lipid for the larval stages of laboratory-reared stable flies, from which they acquire abundant 20:5n-3. The PL fatty acid profiles of the ensuing adults include substantial proportions of 20:5n-3 (>5% of PL fatty acids). It appears that the larval diet influenced the adult fatty acid composition because 20:5n-3 is not present in the diets of the adults. Moreover, unlike the example from tick salivary glands, blood-fed flies did not feature substantially higher proportions of 20:4n-6 than their sugar-fed counterparts. Taken together, the findings from waxmoths, mosquitoes and stable flies indicate that the dietary experiences of insect larvae exert substantial influences on adult fatty acid compositions.

The incorporation of exogenous radioactive C_{20} PUFAs into cellular PLs has been recorded in a few insect species, including the house fly (Wakayama *et al.*, 1985), the field cricket *Teleogryllus commodus* (Stanley-Samuelson *et al.*, 1986), and the mosquito *Aedes aegypti* (Petzel *et al.*, 1993). We recently found that hemocytes from the tobacco hornworm, *Manduca sexta*, express a fatty acid incorporation system that is sensitive to fatty acid structure and PL class (Gadelhak and Stanley-Samuelson, 1994). Stable flies incorporated radioactivity associated with 20:4n-6 and with 18:1n-9 into PLs, DGs and TGs. We infer from the different patterns of incorporation that fatty acid incorporation is regulated with respect to fatty acid structure, as shown in the hornworm.

We also noted substantial age differences in the patterns of arachidonic acid incorporation.

First, it appears that most of the radioactivity recovered from day 1 adults was incorporated into a major lipid class, while most radioactivity recovered from day 5 adults was not incorporated into complex lipids. This is an unusual finding because free fatty acids are not found in abundance in animal tissues. Although the point requires further analysis, this reduced incorporation may be related to sexual maturation. It appears, however, that mature flies do not efficiently incorporate exogenous C_{20} PUFAs into tissue lipids. Again, this stresses the relative importance of the larval stages for acquisition of these essential components. These findings indicate that substrate for eicosanoid biosynthesis is abundantly available in stable fly tissue lipids. Because eicosanoid biosynthesis is thought to be an important element of successful blood feeding by ticks (Sauer *et al.*, 1993), future studies will be aimed at assessing eicosanoid biosynthesis in salivary glands from stable flies.

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